

PATENT
10/001,267
Docket 093/004p

CLAIM AMENDMENTS

1 to 12. *Cancelled*

13. *(Currently amended)* A method for producing differentiated cells from primate pluripotent stem (pPS) cells, comprising:
- a) obtaining a culture of pPS cells;
 - b) ~~optionally~~ initiating differentiation of the pPS cells; and then simultaneously or subsequently
 - c) culturing the cells of step b) in a medium containing a histone deacetylase inhibitor, until at least ~60% of the cultured cells have at least three of the following characteristics:
 - antibody-detectable expression of α_1 -antitrypsin (AAT);
 - antibody-detectable expression of albumin;
 - absence of antibody-detectable expression of α -fetoprotein;
 - RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
 - evidence of glycogen storage;
 - evidence of cytochrome p450 activity;
 - evidence of glucose-6-phosphatase activity; or
 - the morphological features of hepatocytes.
14. *(Previously presented)* The method of claim 13, wherein at least about 60% of the cells have at least five of said characteristics.
15. *(Previously presented)* The method of claim 13, wherein at least about 80% of the cells have at least seven of said characteristics.
16. *(Previously presented)* The method of claim 13, wherein the histone deacetylase inhibitor is n-butyrate.
17. *(Previously presented)* The method of claim 13, wherein the histone deacetylase inhibitor is propionic acid, isovaleric acid, or isobutyric acid.
18. *(Previously presented)* The method of claim 13, wherein the histone deacetylase inhibitor is Trichostatin A.

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19. *(Previously presented)* The method of claim 13, wherein differentiation of the pPS cells is initiated by forming embryoid bodies.
20. *(Previously presented)* The method of claim 13, wherein differentiation of the pPS cells is initiated by culturing in a medium containing dimethyl sulfoxide (DMSO), dimethylacetamide (DMA); hexamethylene bisacetamide, or another polymethylene bisacetamide.
21. *(Previously presented)* The method of claim 13, comprising further culturing the cells in a medium containing a cytokine or hormone selected from glucocorticoids, epidermal growth factor (EGF), insulin, TGF- α , TGF- β , fibroblast growth factor (FGF), hepatocyte growth factor (HGF), IL-1, IL-6, IGF-I, IGF-II, and HBGF-1.
22. *(Previously presented)* The method of claim 21, wherein the cells are cultured in a medium containing at least three of said cytokines or hormones.
23. *(Previously presented)* The method of claim 22, wherein the cells are cultured in a medium containing EGF, TGF- α , and HGF.
24. *(Previously presented)* The method of claim 13, further comprising maintaining the differentiated cells by culturing them in a medium containing a histone deacetylase inhibitor.
25. *(Previously presented)* The method of claim 13, further comprising maintaining the differentiated cells by culturing them in a medium containing n-butyrate.

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26. (Previously presented) The method of claim 27, wherein the pPS cells are human embryonic stem cells.

27. (Currently amended) A method for maintaining hepatocyte lineage cells in culture, comprising:
a) obtaining a population of cells differentiated from an established culture of primate pluripotent stem (pPS) cells, comprising wherein at least ~60% of the differentiated cells have at least three of the following characteristics:

- antibody-detectable expression of α_1 -antitrypsin (AAT);
- antibody-detectable expression of albumin;
- absence of antibody-detectable expression of α -fetoprotein;
- RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
- evidence of glycogen storage;
- evidence of cytochrome p450 activity;
- evidence of glucose-6-phosphatase activity; or
- the morphological features of hepatocytes; and then

b) culturing the differentiated cells in a medium containing a histone deacetylase inhibitor, so that at least ~60% of the cultured cells maintain at least three of the following characteristics:

- ~~antibody-detectable expression of α_1 -antitrypsin (AAT);~~
- ~~antibody-detectable expression of albumin;~~
- ~~absence of antibody-detectable expression of α -fetoprotein;~~
- ~~RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);~~
- ~~evidence of glycogen storage;~~
- ~~evidence of cytochrome p450 activity;~~
- ~~evidence of glucose-6-phosphatase activity; or~~
- ~~the morphological features of hepatocytes~~

said characteristics.

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28. *(Currently amended)* A method for producing differentiated cells from human embryonic stem (hES) cells, comprising:
- a) obtaining a culture of hES cells;
 - b) ~~optionally~~ initiating differentiation of the hES cells; and then simultaneously or subsequently
 - c) culturing the cells of step b) in a medium containing a histone deacetylase inhibitor, until at least ~60% of the cultured cells have at least three of the following characteristics:
 - antibody-detectable expression of α_1 -antitrypsin (AAT);
 - antibody-detectable expression of albumin;
 - absence of antibody-detectable expression of α -fetoprotein;
 - RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
 - evidence of glycogen storage;
 - evidence of cytochrome p450 activity;
 - evidence of glucose-6-phosphatase activity; or
 - the morphological features of hepatocytes.
29. *(Previously presented)* The method of claim 13, wherein the pPS cells are cultured with the histone deacetylase inhibitor without previously initiating differentiation.
30. *(Previously presented)* The method of claim 13, wherein the pPS cells are cultured on an extracellular matrix without feeder cells before contact with the histone deacetylase inhibitor.
31. *(Previously presented)* The method of claim 28, wherein at least about 60% of the cells have at least five of said characteristics.
32. *(Previously presented)* The method of claim 28, wherein at least about 80% of the cells have at least seven of said characteristics.
33. *(Previously presented)* The method of claim 28, wherein the histone deacetylase inhibitor is n-butyrate or Trichostatin A.

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34. *(Previously presented)* The method of claim 28, comprising pre-differentiating the cells by culturing in a medium containing dimethyl sulfoxide (DMSO), dimethylacetamide (DMA); hexmethylene bisacetamide, or another polymethylene bisacetamide.
35. *(Previously presented)* The method of claim 28, comprising further culturing the cells in a medium containing at least three cytokines or hormones selected from glucocorticoids, epidermal growth factor (EGF), insulin, TGF- α , TGF- β , fibroblast growth factor (FGF), hepatocyte growth factor (HGF), IL-1, IL-6, IGF-I, IGF-II, and HBGF-1.
36. *(Previously presented)* The method of claim 34, wherein the cells are cultured in a medium containing EGF, TGF- α , and HGF.

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37. *(Previously presented)* The method of claim 27, wherein at least about 60% of the cells have at least five of said characteristics.
38. *(Previously presented)* The method of claim 27, wherein at least about 80% of the cells have at least seven of said characteristics.
39. *(Previously presented)* The method of claim 27, wherein the histone deacetylase inhibitor is n-butyrate.
40. *(Previously presented)* The method of claim 27, wherein the histone deacetylase inhibitor is Trichostatin A.
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Upon allowance of the application, please renumber the claims as follows:

Claim	13	→	1	Claim	28	→	16
	14	→	2		29	→	7
	15	→	3		30	→	8
	16	→	4		31	→	17
	17	→	5		32	→	18
	18	→	6		33	→	19
	19	→	9		34	→	20
	20	→	10		35	→	21
	21	→	11		36	→	22
	22	→	12		37	→	24
	23	→	13		38	→	25
	24	→	14		39	→	26
	25	→	15		40	→	27
	26	→	28				
	27	→	23				

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